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ORIGINAL ARTICLE

Morphology and morphogenesis of a new oxytrichid ciliate, *Notohymena limus* n. sp. (Ciliophora, Oxytrichidae) from Delhi, India

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Abstract The morphology and morphogenesis of a new oxytrichid ciliate, *Notohymena limus* n. sp. were studied *in vivo* and after protargol impregnation. The new ciliate was isolated from the sewage sludge at Delhi Jal Board Sewage Treatment Plant located at Rithala, Delhi, India, using the non-flooded Petri dish method. *N. limus* n. sp. is characterized as follows: flexible dorsoventrally flattened ellipsoidal body; *Notohymena*-pattern undulating membranes; adoral zone of membranelles (AZM) occupied about 39% of the body length, and consists of around 26 membranelles; large and deep buccal cavity; colorless subpellicular granules present in groups and arranged around the bases of dorsal bristles; 4 macronuclear nodules; 2 micronuclei; 18 fronto-ventral-transverse (FVT) cirri in typical *Oxytricha*-pattern; 6 dorsal rows of bristles; 3 caudal cirri; about 16 right and 15 left marginal cirri; *N. limus* n. sp. is a new species on the basis of the combination of morphological, morphometric and morphogenetic characteristic features.

1. Introduction

The genus *Notohymena* was erected by Blatterer and Foissner (1988). The cells have a strong resemblance to genus *Oxytricha*, the two being almost indistinguishable *in vivo*. Members of this genus are characterized by their highly flexible body, 18 or less fronto-ventral-transverse (FVT) cirri, question mark (?) shaped adoral zone of membranelles (AZM), presence of caudal cirri, undulating membranes in *Notohymena* pattern and one right and one left marginal row of cirri (Berger, 1999; Berger and Foissner, 1997; Blatterer and Foissner, 1988).

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Various species assigned to the genus – *Notohymena rubescens*, Blatterer and Foissner, 1988; *Notohymena australis* (Foissner and O'Donoghue, 1990), Berger, 1999; Voss, 2008; *Notohymena antarctica,* Foissner, 1996; *Notohymena pampasica*, Küppers et al., 2007; *Notohymena saprai*, Kamra and Kumar, 2010; *Notohymena apoaustralis*, Lv et al., 2013; *Notohymena selvatica* (Hemberger, 1985), Blatterer and Foissner, 1988, have overlapping species-level characters with respect to their live morphology, infraciliature, morphometry and habitat. The present study provides detailed morphology and morphogenesis of a new isolate of genus *Notohymena* isolated from a sewage sludge sample to ascertain its taxonomic position.

2. Material and methods

2.1. Sample site and sampling

The Delhi Jal Board distributes potable/usable water after collecting and treating raw water from various sources like River Yamuna, Bhakhra Storage, Ganga Canal and Groundwater. Sewage water from various sources is also collected for treatment and disposal by the Delhi Jal Board Sewage Treatment Plants located in various areas within Delhi. The sewage sludge containing debris, fine sand and litter arises after filtration during the sewage water recycling process which later on dries naturally. This dried sludge sample was collected from Delhi Jal Board Sewage Treatment Plant located at Rithala, Delhi (28°43′32″N, 77°6′18″E). Ciliates were obtained using the non-flooded petridish method (Foissner, 1992, 1987).

2.2. Morphology and morphogenesis

Infraciliature of *Notohymena limus* n. sp. was studied using Protargol impregnation (Kamra and Sapra, 1990; Wilbert, 1975). Counts and measurements of the 20 impregnated specimens were performed at magnification of 1000×. Terminology used is mainly according to Berger (2008, 1999), Foissner and Stoeck (2011) and Küppers et al. (2011). Cirri Numbering system established by Borror (1972), Hemberger (1985), Martin (1982) and Wallengren (1900) is followed.

2.3. pH of the sample

Dried sludge sample was mixed with distilled water in a ratio of 1:2.5 and shaken for at least half an hour. The pH of the supernatant was determined using glass electrode pH meter (Decibel db-1003).

2.4. Percentage of organic matter of sample

The organic matter content of the dried sludge sample was estimated by modified Walkley–Black method (Walkley and Black, 1934).

3. Results

3.1. Occurrence

Ciliate *N. limus* n. sp. was isolated from the dried sludge sample at Delhi Jal Board Sewage Treatment Plant located at Rithala, Delhi. The organic matter content of this sample accounted for 0.32% and the pH was 7.9.

3.2. Characteristic features

Size of protargol impregnated cell about 62 × 22 μm; shape ellipsoidal with anterior rounded and posterior lanceolate end; buccal cavity large and deep; undulating membranes in typical *Notohymena*-pattern; colorless sub-pellicular granules; 4 macronuclear nodules; 2 micronuclei; 18 Frontal-Ventral-Transverse ($F_{1–8}$, $V_{1–5}$, $T_{1–5}$) cirri; on an average 26 adoral membranelles; 16 right and 15 left marginal cirri; 6 dorsal rows of bristles (Dorsal Kineties-DK_{1-4} and Dorso-Marginals-$DM_{1–2}$); 3 caudal cirri (Fig. 1).

3.3. Etymology

The species has been named *limus*, Latin noun for sludge referring to the habitat from where the species was discovered.

3.4. Description of the species

The average size of Protargol impregnated non-dividing cell is 62 × 22 μm. The shape of the cell is ellipsoidal with anterior rounded and posterior lanceolate end. The body is flexible, dorsoventrally flattened with length to width ratio of 2.9:1. The cells exhibited frequent encystment and excystment. Cysts have a smooth surface and measuring about 27.8 μm in diameter in protargol stained preparations.

There are four macronuclear nodules, each measuring about 8 × 7 μm. Two small spherical and compact micronuclei with a diameter about 2.66 μm each are present on variable

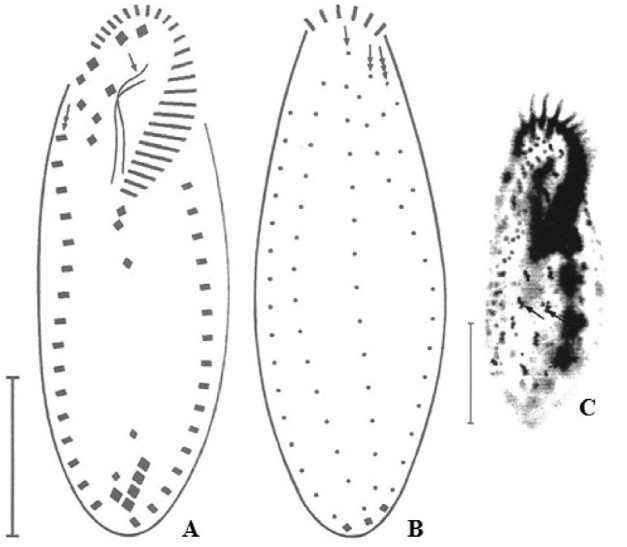

Figure 1 Line diagrams (A, B) and photomicrograph (C) of Protargol impregnated cells of *N. limus* n. sp. showing ventral (A) and dorsal (B, C) surface. (A) UMs in *Notohymena* pattern (arrow), RMC row (double arrow) anteriorly at the level of F_7. (B) Dorsal rows (arrow), DM_1 (double arrow), very small DM_2 (Triple arrow). (C) Granules present in a group of 2–6 at the bases of dorsal bristles (arrows), four macronuclear nodules. Bar represents 20 μm.

positions. Colorless subpellicular granules are present in groups and are arranged around the bases of dorsal bristles. The buccal cavity is large and deep and undulating membranes are in typical *Notohymena* pattern. Adoral zone of membranelles (AZM) is well developed and consists of about 26 membranelles occupying 39% of the body length. The frontal-ventral-transverse (FVT) ciliature consists of 18 FVT cirri. The three post oral ventral cirri appear near the cytostome, with V_1 and V_2 placed as a pair while V_3 is away from them. The two pretransverse ventral cirri are near the transverse cirri. T_{1-4} are arranged in an oblique linear row adjoined by T_5 forming a tick mark pattern. The row of right marginal cirri (RMC) starts at the level of F_7 and ends at the level of the fifth transverse cirrus. The row of left marginal cirri (LMC) curves into 'J' shape towards the mid line of the cell. The dorsal ciliature consists of 6 dorsal rows of bristles. The dorsal kineties, DK_{1-4} are curved and extend along the entire body length. DK_{1-3} are convex in the posterior half of the cell but DK_4 is concave at about the mid body region. The two dorsomarginal (DM) rows are short rows. DM_2 is very short consisting of only one or two bristles. There are three caudal cirri (CC) one each at the ends of $DK_{1,\ 2\ \&\ 4}$.

All morphometric features are shown in Table 1, while a comparison between the morphometric data of the new species *N. limus* n. sp. and *N. saprai* (Kamra and Kumar, 2010) is shown in Table 2.

3.5. Developmental morphogenesis

Stomatogenesis begins with *de-novo* appearance of kinetosomes in the region between V_4 and LMC and above the transverse cirri. Further proliferation leads to the formation of an anarchic field of oral primordium (OP) which in later stages grows in both directions (anteriorly toward the adoral zone and posteriorly toward the transverse cirri). Before elongating anteriorly, it reaches close to the transverse cirri. Two sets of six FVT ciliary streaks develop in the manner as described for sub-family Oxytrichinae (Berger and Foissner, 1997; Berger, 1999; Shao et al., 2015).

In the proter, parental undulating membranes (UMs) function as streak I, while the streak II originates from the disaggregating F_1 and a few anteriorly moved kinetosomes from OP. Streaks III and IV originate from disaggregating F_8 and F_7 respectively. Streak V originates from the anterior movement and splitting of streak V of opisthe. Streak VI originates *de-novo*.

In the opisthe, streak I, II and III originate from the OP. Subsequently, streaks IV, V and VI originate from disaggregating V_1, V_2 and V_3. Kinetosomes from OP and streak V move anteriorly to contribute to the streaks for proter.

Differentiation of the 18 FVT cirri follows the usual Oxytrichinae pattern 1,3,3,3,4,4 for both the daughter cells.

Table 1 Morphometric characterization of *Notohymena limus* n. sp.

Character[a]		Mean	Min	Max	SD	CV	*N*
Body, length		61.50	54.90	74.50	6.27	10.20	20.00
Body, width		21.90	16.90	27.10	2.89	13.22	20.00
Body length/body width		2.90	2.40	3.60	0.36	12.63	20.00
Ma, No.		4.00	4.00	4.00	0.00	0.00	20.00
Ma, length		7.70	5.90	9.40	0.91	11.79	20.00
Ma, width		6.50	6.10	7.70	0.48	7.35	20.00
Mi, No.		2.00	2.00	2.00	0.00	0.00	20.00
Mi, diameter		2.70	2.60	2.80	0.10	3.76	20.00
AM, No.		25.80	22.00	28.00	1.48	5.74	20.00
AZM, length		23.60	19.60	27.80	2.31	9.80	20.00
AZM length/body length		0.40	0.30	0.40	0.03	7.69	20.00
FC, No.		8.00	8.00	8.00	0.00	0.00	20.00
VC, No.		5.00	5.00	5.00	0.00	0.00	20.00
TC, No.		5.00	5.00	5.00	0.00	0.00	20.00
LMC, No.		14.50	13.00	16.00	0.84	5.81	20.00
RMC, No.		15.60	14.00	17.00	1.07	6.87	20.00
DK, No.		4.00	4.00	4.00	0.00	0.00	20.00
DM, No.		2.00	2.00	2.00	0.00	0.00	20.00
DB, No. in	DK_1	15.90	14.00	17.00	1.22	7.70	10.00
	DK_2	15.10	14.00	17.00	1.22	8.06	10.00
	DK_3	13.50	12.00	16.00	1.51	10.36	10.00
	DK_4	14.60	13.00	16.00	1.51	10.36	10.00
	DM_1	4.00	3.00	5.00	0.63	15.75	10.00
	DM_2	1.50	1.00	2.00	0.55	36.67	10.00
Total DB, No.		64.30	50.00	69.00	3.50	5.44	10.00
CC, No.		3.00	3.00	3.00	0.00	0.00	10.00

[a] All data are based on Protargol impregnated specimens. Measurements are in μm. Ma, macronuclear nodules; Mi, micronucleus; AM, adoral membranelles; AZM, adoral zone of membranelles; FC, frontal cirri; VC, ventral cirri; TC, transverse cirri; LMC, left marginal cirri; RMC, right marginal cirri; DK; dorsal kineties; DM, dorsomarginal; DB, dorsal bristles; CC, caudal cirri.
Mean, arithmetic mean; Min, minimum; Max, maximum; CV, coefficient of variation in %; SD, standard deviation; *N*, no of cells.

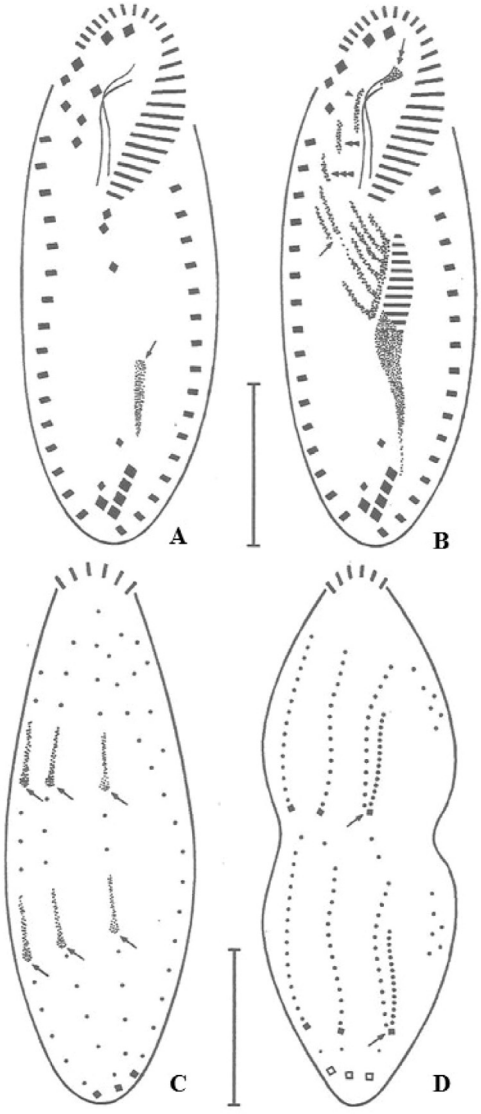

Figure 2 Line diagrams of Protargol impregnated cells of *N. limus* n.sp. showing divisional morphogenesis on the ventral surface (A, B) and dorsal surface (C, D). (A) *De-novo* origin of OP (arrow). (B) Anterior movement of streak V of opisthe (arrow), reorganizing UM (double arrow), streak II formed from F_1 and kinetosomes of OP (arrow head), streak III from F_8 (double arrow head), streak IV from F_7 (triple arrow head). (C) Within row proliferation of two sets of three dorsal primordia and thickening of posterior ends to form caudal cirri (arrows). (D) Formation of new DK_4 after splitting of third dorsal primordia (arrows). Bar represents 20 μm.

Development of the marginal cirri is by within the row primordia formation, a process similar to that described for the family Oxytrichidae (Berger, 1999; Arora et al., 1999; Gupta et al., 2001, 2003, 2002, 2006; Naqvi et al., 2006).

On the dorsal surface, two sets of three dorsal primordia form by within the row primordia formation one each for each daughter cell. The third dorsal primordium, DP_3 splits and forms new DK_3 and DK_4. Two more primordia appear near the RMC row on the ventral surface giving rise to two dorsomarginal rows, which later shift to the dorsal surface (Fig. 2).

Table 2 A comparison between the morphometric features of the new ciliate species *Notohymena limus* n. sp. and *N. saprai* (Kamra and Kumar, 2010).

Character[a]	*N. limus* n. sp. (present study)	*N. saprai* (Kamra and Kumar, 2010)
Cortical granules	Colorless	Dark green
Body, length	61.50	149.20
Body, width	21.90	48.80
AM, No.	25.80	52.70
AZM, length	23.60	–
AZM/body length	40.00	38.50
Ma, No.	4.00	4.00
Ma, length	7.70	13.80
Ma, width	6.50	9.10
Mi, No.	2.00	3.70
Mi, diameter	2.70	2.00
FC, No.	8.00	8.00
VC, No.	5.00	5.00
TC, No.	5.00	5.00
RMC, row	1.00	1.00
RMC, No.	15.60	43.10
LMC, row	1.00	1.00
LMC, No.	14.50	43.90
Dorsal rows, No.	6.00	6.00
DK_1	15.90	30.90
DK_2	15.10	31.10
DK_3	13.50	21.00
DK_4	14.60	12.70
DM_1	4.00	23.10
DM_2	1–2	11.50
CC, No.	3.00	3.00

[a] All data are based on Protargol-impregnated specimens. Measurements in μm. AM, adoral membranelles; AZM, adoral zone of membranelles; Ma, macronuclear nodules; Mi, micronuclei; FC, frontal cirri; VC, ventral cirri; TC, transverse cirri; RMC, right marginal cirri; LMC, left marginal cirri; DK, dorsal kineties; DM, dorsomarginals; CC, caudal cirri.

Comparison of morphogenetic characterization of the closely related species *N. limus* n. sp., *N. saprai* and *N. rubescens* (Blatterer and Foissner, 1988) is shown in Table 3.

4. Discussion

4.1. Occurrence

Notohymena limus n sp. was isolated from the sewage sludge sample at Delhi Jal Board Sewage Treatment Plant, Rithala. The sewage sludge sample was slightly alkaline with high organic carbon content.

4.2. Comparison of Notohymena limus n. sp. with other related species

4.2.1. Morphology and morphometry

Notohymena limus n sp. is distinct from other reported species of the genus that have been described thus far in having a new combination of characters. Though it is similar to *N. saprai* in few characters as both have 4 macronuclei, 3 caudal cirri, 18 FVT cirri and the presence of cortical granules it is distinct

Table 3 Morphogenetic characterization of the most similar species *N. limus* n. sp., *N. saprai* and *N. rubescens*.

Variable	*N. limus* n. sp. (present study)	*N. saprai* (Kamra and Kumar, 2010)	*N. rubescens* (Blatterer and Foissner, 1988)
OP origin	*De-novo* close to V_4 and transverse cirri	*De-novo* close to V_1, V_2 and V_3	Transverse cirri
Proter			
I	Parental UM	Parental UM	Parental UM
II	II/2 (F_1) and OP	II/2 (F_1)	II/2 (F_1)
III	III/2 (F_8)	III/2 (F_8)	III/2 (F_8)
IV	IV/3 (F_7)	IV/3 (F_7)	IV/3 (F_7)
V	Splitting of primary primordia from opisthe	OP	*De-novo*
VI	*De-novo*	OP	*De-novo*
Opisthe			
I	OP	OP	OP
II	OP	OP	–
III	OP	OP	–
IV	IV/2 (V_1)	OP (POVC incorporated)	–
V	V/4 (V_2)	OP	–
VI	V/3 (V_3)	OP	–

OP: oral primordium; UM: undulating membranes.

in other morphometric characters such as: colorless granules, size, AZM number, AZM/body length (BL), macronuclei length and width, number of micronuclei and number of cilia in dorsal rows (Tables 1 and 2).

4.2.2. Morphogenesis

The reorganization of the undulating membranes corresponds to that in *N. australis, N. rubescens* and other members of the family. The genus specific hook at the anterior end of the paroral is formed by addition of basal bodies whereby the undulating membranes are arranged side by side (Berger, 1999). They cross (optically) in late stages and attain the genus specific pattern shortly after cell division similar to *Cyrtohymena* and *Steinia*, suggesting that this character is a young evolutionary acquisition (Voss, 1991a,b; Voss and Foissner, 1996).

The detailed morphogenesis of *N. limus* n. sp. described in the present investigation shows that its process of stomatogenesis is distinct from other reported species of the genus. In this species, stomatogenesis begins with the *de-novo* appearance of kinetosomes in the region between V_4 and LMC (Left marginal cirri) and above the transverse cirri which is not in accordance with the observations in *N. rubescens* (OP is formed close to the uppermost transverse cirrus T_1; Berger, 1999) and *N. saprai* (OP arises close to three POVC; Kamra and Kumar, 2010). The origin of FVT primordia also show slight variations from the previously described other species of *Notohymena* (Table 3).

5. Conclusion

The ciliate *N. limus* n. sp. isolated from the sewage sludge sample at the Delhi Jal Board Sewage Treatment Plant, Rithala, is a distinctly new species on the basis of its morphological, morphometric and morphogenetic features.

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